

EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Editors

Ibrahim Ali Noorbacha

Mohamed Ismail Abdul Karim

Hamzah Mohd Salleh

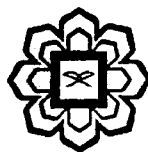


IIUM Press

EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Editors

Ibrahim Ali Noorbatcha
Mohamed Ismail Abdul Karim
Hamzah Mohd Salleh



IIUM Press

Published by:
IIUM Press
International Islamic University Malaysia

First Edition, 2011
©IIUM Press, IIUM

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without any prior written permission of the publisher.

Perpustakaan Negara Malay Cataloguing-in-Publication Data

Ibrahim Ali Noorbatcha, Mohamed Ismail Abdul Karim and Hamzah Mohd Salleh
Experimental Methods in Modern Biotechnology

ISBN: 978-967-0225-86-9

Member of Majlis Penerbitan Ilmiah Malaysia – MAPIM
(Malaysian Scholarly Publishing Council)

Printed by:
IIUM PRINTING SDN. BHD.
No. 1, Jalan Industri Batu Caves 1/3
Taman Perindustrian Batu Caves
Batu Caves Centre Point
68100 Batu Caves
Selangor Darul Ehsan

CONTENTS

Preface

Chapter 1. Immobilization of Enzymes

Faridah Yusof

1.	Introduction	1
2.	Scope of This Chapter	1
3.	Salient Features of Enzyme Immobilization	1
4.	Advantages of Enzyme Immobilization	2
5.	Disadvantages of Enzyme Immobilization	2
6.	Methods of enzyme immobilization	2
6.1	Adsorption Method	2
6.2	Covalent Bonding Method	3
6.3	Entrapment Method	3
6.4	Copolymerization or Cross-Linking Method	4
6.5	Encapsulation Method	4
6.6	Carrier-Free Enzyme Immobilization	4
6.6.1	Cross-Linked Dissolved Enzymes (CLE)	4
6.6.2	Cross-Linked Enzyme Crystals (CLEC)	5
6.6.3	Cross-Linked Enzyme Aggregates (CLEA)	5
7.	Materials and Methods	5
7.1	Immobilization of α -amylase by Entrapment	6
7.2	Carrier-free immobilized enzymes - Cross-Linked Enzyme Aggregates (CLEA)	7
8.	Notes and Tips	10
9.	References	10
10.	Further Readings	10

Chapter 2. Protein Extraction and Purification

Faridah Yusof

1.	Introduction	11
2.	Scope of This Chapter	11
3.	Approaches to Protein Purification	12
3.1	Development of Assay for the Protein	12
3.2	Extraction of Protein from Sources	12
3.3	Fractionation of Proteins	13
3.3.1	Types of Column Chromatography Techniques	15

3.3.1.1	Ion-Exchange Chromatography	16
3.3.1.2	Gel Filtration Chromatography	16
3.3.1.3	Hydrophobic Interaction Chromatography	17
3.3.1.4	Chromatofocussing	17
3.3.1.5	Affinity Chromatography	18
4.	Determination of Protein Purity	18
5.	Purification of an ‘Inhibitor’ Protein from <i>Hevea brasiliensis</i> Latex	19
5.1	Materials and Methods	19
5.1.1	Materials	19
5.1.2	Methods	19
5.1.2.1	Development of Assay for the ‘Inhibitor’ Protein	19
5.1.2.2	Extraction of Crude Protein from Fresh Latex	19
5.1.2.3	Fractionation of C-serum Protein by Column	19
5.1.2.4	Determination of Purity of ‘Inhibitor’ Protein	21
5.2	Results and Discussion	22
6.	Notes and Tips	22
7.	Summary	23
8.	References	24
9.	Further Readings	24

Chapter 3. Methods for Screening the Potential Natural Compound for Treating Metabolic Disorder Diseases: Anti-Inflammatory and Griess Assay [Nitric Oxide (NO) Measurement]

Azura Amid, Sulawatie Semail, Wan Dalila Wan Chik and Hammed Ademola Monsur

1.	Introduction	25
2.	Objective	25
3.	Materials	26
4.	Methods	27
4.1	Preparation of 1L cell culture media	27
4.2	Preparation of media with 10% fetal bovine serum (FBS)	28
4.3	Preparation of 1L PBS-EDTA	28
4.4	Resuscitation of Frozen Cell Lines	28
4.5	Subculture of Adherent Cell Lines	28
4.6	Cells Quantification	29
4.7	Procedure to treat RAW 264.7 macrophage cells with identified extract	29
4.8	Preparation of nitrite standards curve	30
4.9	Griess Reaction	30
4.10	Nitrite Concentration Determination	30
5.	Example of Results Obtained	31
6.	References	33
7.	Further Reading	34

Chapter 4. Factors Affecting Enzyme Assays

Hamzah Mohd Salleh

1.	Introduction to Enzyme Assay	35
	1.1 Factors That Affect Enzyme Activity	35
2.	Objective of The Experiments	36
3.	Materials	36
4.	Enzyme Lab 1	37
	4.1 Effect of pH on Enzyme Activity	37
	4.1.1 Procedure	38
5.	Enzyme Lab 2	39
	5.1 Effect of Temperature on Enzyme Activity and Enzyme Stability	39
	5.1.1 Procedure “A”.	39
	5.1.2 Procedure “B”.	39
6.	Enzyme lab 3	40
	6.1 Effect of Substrate Concentration on Enzyme Rates	40
	6.1.1 Procedure	42
7.	For Further Readings	45
8.	Appendix	46

Chapter 5. Techniques of Extraction and Purification of Fucoxanthin from Brown Seaweeds

*Irwandi Jaswir, Dedi Noviendri, Hamzah Mohd Salleh, Muhammad Taher and
Kazuo Miyashita*

1.	Introduction	50
2.	Materials	51
3.	Methods	51
	3.1 Extraction of Fucoxanthin	51
	3.2 Purification of Fucoxanthin (with SiO ₂ Open Column Chromatography)	52
	3.3 Further Purification of Fucoxanthin (with ODS Double Column)	52
	3.4 Ilustration of Extraction and Purification of Fucoxanthin from Brown Seaweeds	55
4.	Notes	57
5.	Acknowledgments	59
6.	References	59
7.	Appendix	62

Chapter 6. Fundamentals of Proximate Analysis in Food Products

Irwandi Jaswir and Asiyambi-Hammed Tawakalit Tope

1.	Introduction	65
2	Determination of Moisture and Total Solids	66
2.1	Sample Preparation	66
2.2	Evaporation Methods	66
2.3	Distillation Methods (Dean and Stark Method)	67
2.4	Chemical Reaction Methods	67
2.4.1	<i>Karl-Fisher</i> method	67
2.4.2	Gas production methods	68
3.	Analysis Of Ash Content	69
3.1	Sample Preparation	69
3.2	Dry Ashing	69
3.3	Wet Ashing	70
4.	Analysis of Fats	71
4.1	Introduction	71
4.2	Sample Selection and Preservation	71
4.3	Determination of Total Fats Content	71
4.3.1	Solvent Extraction	72
4.3.1.1	Continuous Solvent Extraction Method:Goldfish Method	72
4.3.1.2	Semicontinuous Solvent Extraction Method: Soxhlet Method	72
4.3.2	Non solvent Liquid Extraction Methods	73
4.3.2.1	<i>Babcock</i> Method	73
4.3.2.2	<i>Gerber</i> Method	73
4.3.2.3	<i>Detergent</i> Method	73
4.3.3	Instrumental methods.	73
5.	Analysis of Proteins	74
5.1	Determination of Overall Protein Concentration	74
5.1.1	Kjeldahl method	74
5.1.1.1	<i>Digestion</i>	74
5.1.1.2	<i>Neutralization</i>	75
5.1.1.3	<i>Titration</i>	75
5.1.2	Enhanced Dumas method	75
5.1.3	Methods using UV-visible spectroscopy	76
5.1.3.1	Direct measurement at 280nm (Absorption Method)	77
5.1.3.2	<i>Biuret</i> Method	77
5.1.3.3	<i>Lowry</i> Method	77
5.1.3.4	<i>Dye binding</i> methods	78
5.1.3.5	<i>Turbimetric</i> method	78
6.	Analysis of Carbohydrates	78
7.	Analysis of Fiber	79
7.1	Common Procedures of Sample Preparation	79
7.2	Fiber Determination Method	79

7.2.1 Gravimetric Methods	79
7.2.1.1 Crude Fiber Method	79
7.2.1.2 Total, Insoluble and Soluble Fiber Method	7
7.2.1.3 Chemical Methods	80
8. References	80

Chapter 7. Fish Gelatin Production: Extraction Method and Quality Analysis

Irwandi Jaswir, Hammed A. Monsur and Hamzah M. Salleh

1. Introduction	81
2. Extraction of Gelatin	82
2.1 Materials	82
2.2 Methods	82
2.2.1 Skin preparation	82
2.2.2 Pretreatment	83
2.2.3 Extraction	84
2.2.4 Dehydration	84
3. Quantitative Analysis	87
3.1 Gravimetric method	87
3.2 Soluble protein content method	87
4. Quality Analysis	88
4.1 Preparation of matured gelatin gel	89
4.2 Gel Strength: definition and determination	89
4.3 Measurement of Rheological Parameters	90
4.4 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS- PAGE)	91
4.5 The Foam test (Foam capacity and stability)	91
4.6 Amino acid profiling	92
4.7 Analysis of Color, Turbidity and clarity	92
5. References	93

Chapter 8. Application of Fourier Transform Infrared Spectroscopy Edible Fats and Oils Analysis

Mohamed Elwathig Saeed Mirghani

1. Introduction	96
1.2 Analytical and Quality Control	97
1.3 Fourier Transform Infrared (FTIR) Spectroscopy	97
1.4 Fourier Transformation	98
1.5 Transmission technique	98
1.6 Attenuated total reflectance (ATR)	99
2. Methodology	99
2.1 Sample preparations	100
2.1.1 Gases Samples	100

2.1.2 Liquid samples	100
2.1.3 Solid samples	100
2.2 FTIR Data Handling Techniques	100
2.2.1 Detection of overlapped bands	100
2.2.2 Smoothing and interpolation	100
2.2.3 Baseline correction	101
2.2.4 Peak intensity measurements	101
2.2.5 Spectral stripping (Subtraction)	101
2.2.6 Ratio method	101
3. Quantitative Analysis	102
3.1 Beer-Lambert law	102
3.2 Classical least squares (K – Matrix)	103
3.3 Inverse least squares (P – Matrix)	103
3.4 Partial least square (PLS)	103
3.5 Principal component regression (PCR)	103
4. FTIR Spectroscopy Applications On Food And Lipids	104
4.1 Examples	106
4.1.1 Slip melting point	106
4.1.2 Residual soap in oil	106
4.1.3 Hexane in solvent extracted oil	106
4.1.4 Palm carotene	107
4.1.5 Sesamol in sesame seed oil	107
4.1.6 Gossypol in cottonseed oil	107
4.1.7 Aflatoxins	107
4.1.8 Animal fats	108
4.1.9 Adulteration of edible fats and oils	108
5. References	109

Chapter 9. *In Vitro* Assay for Investigating Potential Anti-Cancer Agents Targeting at Metastatic Level

Yumi Zuhanis Has-Yun Hashim and Chris I.R. Gill

1. Introduction	115
2. Scope	115
3. Material	116
3.1 Equipment/Apparatus/Softwares	116
4. Preparation	117
4.1 Methods	117
5. Notes	118
6. Reference	120
7. List of Abbreviation	122

Chapter 10. Homology Modelling Of Pyranose-2-Oxidase from Phanerochaete Chrysosporium

Ibrahim Ali Noorbacha, Azratul Ashimah Nur Mohd Dom, Ahmad Sidqi Harithuddin and Hamzah Mohd Salleh

1.	Introduction	123
2.	Literature Review	124
	2.1 Biofuel and enzymatic biofuel cells	124
	2.2 Glucose oxidase	124
	2.3 Pyranose-2-oxidase	125
	2.4 Homology Modelling	125
3.	Materials and Methods	126
	3.1 Homology project design	126
	3.2 Homology modeling	126
4.	Result and Discussion	131
	4.1 Description of the results pairwise sequence	133
	4.2 Description of result pairwise sequence alignment	162
	4.3 Ramachandran plot statistics	139
5.	Structure Validation	140
6.	References	141

Chapter 11. Liquid-Liquid Extraction and its Application for Separation of Organic Acids

Parveen Jamal

1.	Introduction	143
	1.1 Liquid-Liquid Extraction	143
	1.2 Principle of Extraction from Liquids	143
2.	Notes and Tips	144
	2.1 Solvent Selection	144
	2.2 Properties of a good solvent	145
	2.3 Precautions	145
	2.4 Class of Organic Compounds and Solubility factor	145
	2.5 Principle of Extracting Different Compounds	146
	2.6 Salting Out	148
3.	Experimental Part: Separation of acid from a mixture containing acid, base and	149
	3.1 Purpose	149
	3.2 Learning Objectives	149
	3.3 Techniques	149
4.	Materials	149
5.	Procedure	150
	5.1 Flow chart of Experimental Procedure	150
	5.2 Suggested Timetable	150
	5.3 Section A: Extraction of the Acid	151

5.4 Section B: Isolation of the Acid	151
5.5 Section C: Recrystallization of the Acid	152
6. Results	152
6.1 Percent Yield Calculation	152
6.2 Example of Data calculation	153
6.3 Calculation	153
7. References	154

Chapter 12. Response Surface Methodology (RSM) Design for Bioreactor Operation

Maizirwan Mel and Najiah Nadir

1. Introduction	155
1.1 Design of Experiment	155
1.2 Response Surface Methodology	155
1.3 Central Composite Design	155
1.4 Box-Behnken Design	156
1.5 Analysis of Variance (ANOVA)	156
1.6 Graphical Analysis	157
1.7 Bioreactor Operation	157
2. Methodology	158
2.1 Preparation of Bioreactor	158
2.1.1 Optimization of Fermentation Parameters for Maximum Ethanol	158
2.1.2 Optimization of Dyestuff Adsorption From Aqueous Solution Using	159
2.2 Experimental Design	159
2.2.1 Central composite design	159
2.2.2 Box-Behnken Design	161
3. Results and Discussion	161
3.1 Regression Equation	161
3.1.1 Central composite design	161
3.1.2 Box-behnken design	162
3.2 Analysis	163
3.2.1 Central composite design	163
3.2.2 Box-Behnken Design	164
3.3 Response Surface Curves	164
3.3.1 Central composite design	164
3.3.2 Box-Behnken Design	166
4. Conclusion	168
5. References	168

Chapter 13. Screening Natural Compounds for Antibacterial Activity by Disc Diffusion Method

Raha Ahmad Raus

1.	Introduction	170
2.	Materials	172
3.	Methods	172
	3.1 Prepare bacterial inoculums	172
	3.2 Adjust inoculums turbidity	172
	3.3 Plating bacterial inoculums on plate	173
	3.4 Place disc on plate	173
	3.5 Measure inhibition zone and interpretation	173
4.	Notes	175
5.	References	175

Chapter 14. Indicator Microorganisms: Detection of *Coliform* and *Escherichia coli*

Mohamed Ismail Abdul Karim

1.	Introduction	176
	1.1 Enumeration Methods	177
	1.2 Solid Media	177
2.	Equipment and materials that are needed are as follows	177
3.	Media and Reagents	178
4.	Conventional Method for testing growth of coliforms, fecal coliforms and <i>E.</i>	178
	4.1 MPN – Presumptive test for coliforms, fecal coliforms and <i>E.coli</i>	178
	4.2 MPN - Confirmed test for coliforms	179
	4.3 MPN - Confirmed test for fecal coliforms and <i>E. coli</i>	180
	4.4 MPN - Completed test for <i>E. coli</i> .	180
	4.5 The IMViC Tests	181
	4.6 Solid medium method using Red Bile Agar - coliforms	182
	4.7 Membrane Filtration (MF) Method - coliforms	183
5.	LST-MUG Method Used for Detecting <i>E. coli</i> in Chilled or Frozen Foods	183
6.	References	184
7.	Appendix	186

Chapter 15. Direct Nucleation Control: A Novel Approach for the Control of Crystal Size Distribution in Crystallization Processes

Mohd Rushdi Abu Bakar, Zoltan Karman Nagy, Ali Nauman Saleemi and Christopher David Rielly

1.	Introduction	190
2.	Materials	192
3.	Methods	193
	3.1 Uncontrolled Anti-solvent Addition	193
	3.2 DNC by Anti-solvent/Solvent Addition	194
4.	Notes	194

4.1 Uncontrolled Anti-solvent Addition	194
4.2 DNC by Anti-solvent/Solvent Addition	194
5. References	197
6. Further Reading	198
7. List of Abbreviation	198